

Effect of domestic sewage on growth of phytoplankton—mathematical model

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Abstract— A mathematical model relating to the change in phytoplankton biomass in the period of growth and nutrient concentration in the media was proposed on the basis of the Monod equation and was testified by simulation experiments. Analysis of the experiment data showed that: the half-saturation constants for growth K_p ($\mu\text{mol/L}$) for *Skeletonema*, *Chaetoceros* and *Prorocentrum* were 5.52, 1.90 and 0.46, respectively; the balance between stimulation of nutrients and the inhibition of some other materials was found in the effect of domestic sewage on algal growth and the stimulation played a leading role; domestic sewage was more stimulative on dinoflagellate than on diatom and chlorophyte when the assemblage of the algae was cultured. The experiment suggested that mathematical model reasonably explained the characteristics of phytoplankton growth in different nutrient conditions and was worthy to be further improved for eutrophication prediction in off-shore water.

Keywords: domestic sewage; phytoplankton; eutrophication nutrient; mathematical model.

INTRODUCTION

It was of great importance for marine environmental management to establish model for prediction of eutrophication and red tide in the off-shore area. Several mathematical models relating to phytoplankton growth and nutrient concentration in the media have been proposed in the previous reports. For example, the OECD model describing the relationship between chlorophyll content and phosphorus load in the surface water was applied to eutrophication prediction in phosphorus-limited estuaries (Wu, 1988), and many multi-regressional equations with several variables based on observation in situ for several years were applied to prediction of red tide by Japanese researchers (Kato, 1985; Ouchi, 1982; 1983). By laboratory simulation experiments, some scientists proposed kinetic models for phytoplankton growth which could

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be used to study the physiological and ecological characteristics of phytoplankton. In this paper, a mathematical model relation to the change in phytoplankton biomass in the period of growth and nutrient concentration in the media was proposed on the basis of the kinetic model established by predecessors and was testified by experiments to provide an elementary study on eutrophication prediction.

ESTABLISHMENT OF THE MATHEMATICAL MODEL

Strictly speaking, the Monod Equation was derived from Michaelis-Menten equation only under the condition of steady-state which was not easy to find in the field. However, phytoplankton growth could be approximately treated as steady-state growth in the period when the environmental factors were relatively stable.

The Monod Equation would be as follows if nutrients (N and P) acted as controlling factors:

$$u = u_{\max}(T, I) \cdot N/(K_N + N) \cdot P/(K_P + P),$$

where u_{\max} was a constant in the laboratory environment with constant T and I . Suppose biomass was Q , then the specific growth rate $u = dQ/(Qdt)$, Monod Equation could be written as:

$$dQ/(Qdt) = u_{\max} \cdot N/((K_N + N) \cdot P/(K_P + P)).$$

Within a unit time (one day), concentration of N and P in the media approximately kept constant (average concentration of the period). When integrated, the above equation became

$$\begin{aligned} \ln Q_t/Q_0 &= u_{\max} \cdot \sum_{i=0}^{t-1} N_i/((K_N + N_i) \cdot P_i/(K_P + P_i)), i = 0, 1, 2, \dots \text{ or} \\ \ln Q_t &= u_{\max} \cdot \sum_{i=0}^{t-1} N_i/((K_N + N_i) \cdot P_i/(K_P + P_i)) + C, \end{aligned} \quad (1)$$

where Q_0 =initial biomass; Q_t =biomass at day t ;

N_i and P_i =concentration of N and P in the media at day $(t - 1)$; C =constant.

Being non-linear, Equation (1) needed to be modified to apply contently in data treatment. It was known from the characteristics of the Monod Equation (Fig.1) that u was theoretically proportional to P (N) while $P \ll K_P$ or $N \ll K_N$. However, no matter how K_N and u_{\max} changed, first approximation could be made provided that nutrient concentration in the media was less than that for maximum growth, thus u was proportional to nutrient concentration i.e., Equation (1) could be approximately treated as:

$$\ln Q_t = K_a \cdot \sum_{i=0}^{t-1} S_i + C. \quad (2)$$

It could be seen from the relationship between K'_a and K_a that K'_a was a corrected constant of K_a by the concentration of N and P. It represented the comprehensive effect on phytoplankton growth of the other factors in the system except the nutrient one. The characteristics of phytoplankton growth could be understood by comparing the values of K_a and K'_a under given conditions via analysing the data on the basis of Equation (2). Described the relationship between biomass and nutrient concentration in Equation (2), it was possible to predict eutrophication by determining nutrient concentrations in the investigated area.

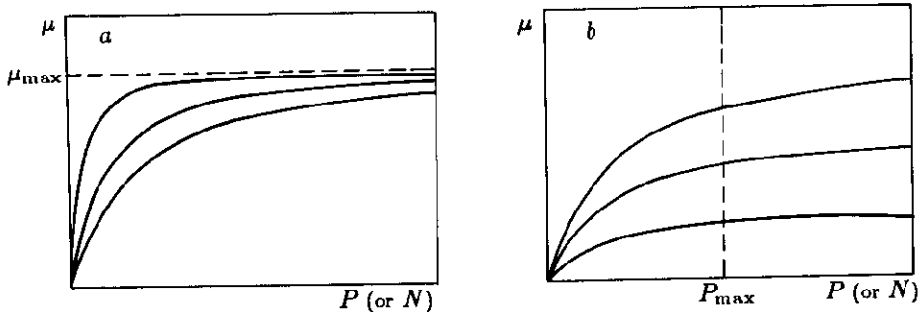


Fig. 1 Relationship between u and P (N)

a: identical u_{\max} and different K_N ; b: identical K_N and different u_{\max}

TESTIFICATION AND APPLICATION OF THE MODEL

K_P values for different algae

Sk., Ch. and Pr. were inoculated respectively in the media with a ratio of DIN:DRP 100 (by atom, DIN=250 $\mu\text{mol/L}$) and cultured in a semicontinuous way ($D=1/5 \text{ day}^{-1}$, to keep the nutrient concentration in constant). The biomass and DRP concentration in the media were determined daily. Because DIN content was rather high, Equation (1) could be approximated to:

$$\ln Q_T = u_{\max} \cdot \sum_{i=0}^{t-1} P_i / (K_P + P_i) + C,$$

where P_i was the real concentration of DRP in the media every day after replenishing the media with nutrients.

u_{\max} was estimated roughly first for calculating the half-saturation constant K_P . u_{\max} was found from the experiment of algae culture in different DRP contents where growth rate (u) for Ch. and Sk. increased as the initial concentration of DRP in the media (C_P^0) increased, and u_{\max} was calculated from the relationship between u and C_P^0 which conformed to $u = u_{\max} \cdot C_P^0 / (K + C_P^0)$, where u_{\max} was the value of u at C_P^0 . Then the u_{\max} was converted to the value at the condition with the same dilution rate in this experiment. For Pr., growth rate at the exponential growth period in the media with different DRP content ($D = 1/5 \text{ d}^{-1}$) was

determined. Where S_i stood for concentrations of N and P in a certain species or the product of them, and the slope K_a was defined as "apparent conditional growth constant", which represented the effect of one or several species of nutrient on phytoplankton growth at the given T and I at constant concentration of several or one species of the coexisting nutrients. A further discussion on K_a in Equation (2) is as follows (only inorganic nutrients were considered in our model, all of the other factors were treated as the term of error). Equation (1) was discussed under the following conditions:

(1) It was supposed that the value of $N_i/(K_N + N_i)$ had little change and only P was considered, then Equation (1) could be approximated to:

$$\ln Q_t = u_{\max} \cdot \bar{N}/(K_N + \bar{N}) \cdot \sum_{i=0}^{t-1} P_i/(K_P + P_i) + C,$$

where \bar{N} was the average concentration of N (and \bar{P} was the average concentration of P). In reference to the characteristics of u -P curve (Fig. 1), $K_P + P_i = K_P + \bar{P}$ could be deduced and above equation further approximated to:

$$\begin{aligned} \ln Q_t &= u_{\max} \cdot \bar{N}/(K_N + \bar{N}) \cdot 1/(K_P + \bar{P}) \cdot \sum_{i=0}^{t-1} P_i + C \\ &= K'_a \cdot \bar{N}/(K_N + \bar{N}) \cdot 1/(K_P + \bar{P}) \cdot \sum_{i=0}^{t-1} P_i + C = K_a \cdot \sum_{i=0}^{t-1} P_i + C, \end{aligned}$$

where K'_a was a constant corresponding to u_{\max} .

(2) Similarly, if only N was considered, then

$$\ln Q_t = K'_a/(K_N + \bar{N}) \cdot \bar{P}/(K_P + \bar{P}) \cdot \sum_{i=0}^{t-1} N_i + C.$$

(3) If N and P were considered simultaneously, then

$$\ln Q_t = K'_a/(K_N + \bar{N}) \cdot 1/(K_P + \bar{P}) \cdot \sum_{i=0}^{t-1} (N_i \cdot P_i) + C.$$

The result was shown in Table 1. It was assumed that $u_{\max} = 0.16 \text{ d}^{-1}$.

Table 1 Growth rate of Pr. in the media with different initial DRP content

$C_P, \mu\text{mol/L}$	2.5	4.2	5.9	7.6	9.3	11.1
u, d^{-1}	0.13	0.16	0.16	0.16	0.16	0.14

At first, the initial value for K_P was obtained by linear regression for $\ln Q_t \sim \sum_{i=0}^{t-1} P_i$ on the supposition that $K_P \gg P_i$ and this K_P was substituted into $\ln Q_t = u_{\max} \cdot \sum_{i=0}^{t-1} P_i / (K_P + P_i) + C$, then stepwise regression was performed with computer to obtain the final value of K_P (Table 2). Although the value of u_{\max} in Table 2 were roughly estimated, they still reflected the growth characteristics of these algae in the view of their order of magnitude, and the value of K_P were also reasonable. The trend was conformable to the situation in the field sea water. The order of the values in Table 2 suggested that the adaptation ability to the environment of low DRP level had a tendency of Pr., Ch. and Sk.. It also could be seen from Table 2 that the high u_{\max} for Sk. and low K_P for Pr. brought ecological advantage of these two red tide species of algae in Xiamen Harbor.

Table 2 u_{\max} and K_P for several algae

Species	u_{\max}, d^{-1}	$K_P, \mu\text{mol/L}$	r	n
Sk.	1.97	5.52	0.986	5
Ch.	0.87	1.90	0.965	5
Pr.	0.16	0.46	0.986	5

Effect of nutrients on the growth of different species of phytoplankton

Phaeodactylum tricornutum was inoculated in the media with nitrogen source of $\text{CH}_2\text{NH}_2\text{-COOH}$ or NH_4Cl and phosphorus source of ATP or KH_2PO_4 respectively. Regression analysis was performed for inorganic nitrogen and phosphorus on the basis of Equation (2). We got the relative order of K'_a under certain conditions, which required nothing but rough estimation for K_P and K_N . The values of K_P for the three algae in Table 2 were used. During the period of experiment, it could be found that the algae showed optimum growth when the ratio of DIN:DRP (in atom) in the media was near to 16:1 (in agreement with Redfield ratio). Then K_N was estimated from $K_N = 16K_P$ as follows: 88-Sk., 30-Ch., and 8-Pr.. Since Sk. and Pr. were relatively particular in their physiological characteristics and the growth model of Ph. and Pl. inoculated in the experiment was similar to that of Ch., we applied K_P and K_N for Ch. to Ph. approximately. The regression result for the experiment data was shown in Table 3.

It was seen from Table 3 that there was generally close correlation of algal growth with inorganic nitrogen as well as inorganic phosphorus. The initial contents of NH_4^+ in the media were near to those of NO_3^- , but the K_a value of NH_4^+ was greater than that for NO_3^- , which suggested that the algae assimilated NH_4^+ preferentially during the growth. The similarity of the K_a value calculated from the terms of DIN DRP, DRP and DIN in each culture system after correction showed that calculated and correction for the parameters were reasonable,

since K'_a was a constant exclusive of the factor of nutrient concentration as mentioned above. By comparing the values for K'_a of different culture systems. It was known that there was similarity between 2# and 4# and between 1# and 3#, which demonstrated that there was little difference in using NH_4Cl and $\text{CH}_2\text{NH}_2\text{COOH}$ as nitrogen source for algal growth, or rather, the decomposition process of $\text{CH}_2\text{NH}_2\text{COOH}$ had little impact on algal growth. K'_a for 1# and 3# were obviously higher than those for 2# and 4#, indicating the stimulative effect of ATP on algal growth when DRP was deficient.

Table 3 Regression for biomass vs. concentration of inorganic N and P in the culture of Ph

Nutrient	K_a	1* (addition of $\text{CH}_2\text{NH}_2\text{COOH}$, ATP)					K'_a
		$r(n=7)$	\bar{N}	$\frac{1}{K_N+\bar{N}}$	\bar{P}	$\frac{1}{K_P+\bar{P}}$	
DRP × DRP	0.036	0.899					4.9
DRP	1.17	0.821	34.0				4.7
DIN	0.007	0.904		0.0156			4.2
NO_3^-	0.009	0.874			0.23		
NH_4^+	0.026	0.965				0.469	
Nutrient	K_a	2* (addition of $\text{CH}_2\text{NH}_2\text{COOH}$, KH_2PO_4)					K'_a
		$r(n=7)$	\bar{N}	$\frac{1}{K_N+\bar{N}}$	\bar{P}	$\frac{1}{K_P+\bar{P}}$	
DIN × DRP	0.009	0.992					1.5
DRP	0.31	0.945	26.6				1.9
DIN	0.011	0.971		0.0177			1.8
NO_3^-	0.014	0.949			1.04		
NH_4^+	0.033	0.979				0.340	
Nutrient	K_a	3* (addition of NH_4Cl , ATP)					K'_a
		$r(n=7)$	\bar{N}	$\frac{1}{K_N+\bar{N}}$	\bar{P}	$\frac{1}{K_P+\bar{P}}$	
DIN × DRP	0.028	0.953					3.9
DRP	1.21	0.881	34.2				4.9
DIN	0.008	0.925		0.0156			4.3
NO_3^-	0.011	0.902			0.026		
NH_4^+	0.027	0.964				0.463	
Nutrient	K_a	4* (addition of NH_4Cl , KH_2PO_4)					K'_a
		$r(n=7)$	\bar{N}	$\frac{1}{K_N+\bar{N}}$	\bar{P}	$\frac{1}{K_P+\bar{P}}$	
DIN × DRP	0.007	0.994					1.3
DRP	0.28	0.957	30.7				1.7
DIN	0.010	0.962		0.0165			1.6
NO_3^-	0.014	0.950			1.18		
NH_4^+	0.029	0.976				0.325	

Effect of domestic sewage on the growth of Sk. and natural phytoplankton population

Natural phytoplankton population collected from Xiamen Harbor, May 15, 1987 and Sk. were inoculated respectively in two experiment runs in four culture media containing the mixture of filtered seawater and filtered domestic sewage with sewage content of 0(1#), 1%(2#),

10%(3[#]) and 50%(4[#]). Regression was performed for biomass of algae vs. nutrient concentration on the basis of Equation (2) and the results were listed in Table 4 and 5. It could be seen from Table 4 and 5 that there was a close correlation of algal growth with inorganic N or P. Comparing the values of K_a and K'_a for N and P in different species, it could be found that:

(1) Except for 4[#] (NH_4^+) \gg (NO_3^-), K_a for NH_4^+ was generally greater than that for NO_3^- , which suggested that $\text{NH}_4^+ - \text{N}$ in sewage was the main N source for algae.

(2) K'_a decreased obviously as sewage content in media increased, which demonstrated that some materials inhibiting algal growth were contained in domestic sewage. Therefore, the biological effect of domestic sewage was of duality, i.e., stimulation due to the increase of nutrient content and inhibition of some materials, in which the former played a leading role and thus algal growth was promoted as a net result.

Table 4 Regression for biomass of Sk. vs. nutrient concentration

Nutrient	K_a	$r(n=4)$	\bar{N}	1 [#] (0)			K'_a
				$\frac{1}{K_N + \bar{N}}$	P	$\frac{1}{K_P + \bar{P}}$	
DIN × DRP	0.038	0.992					27.8
DRP	1.57	0.995	37.1				31.0
DIN	0.009	0.940		7.99×10^{-3}			18.3
NO_3^-	0.010	0.932			0.36		
NH_4^+	0.123	0.992				0.171	
Nutrient	K_a	$r(n=7)$	\bar{N}	2 [#] (1%)			K'_a
				$\frac{1}{K_N + \bar{N}}$	P	$\frac{1}{K_P + \bar{P}}$	
DIN × DRP	0.028	0.878					20.0
DRP	1.26	0.919	35.5				25.3
DIN	0.007	0.988		8.10×10^{-3}			17.2
NO_3^-	0.008	0.987			0.29		
NH_4^+	0.127	0.897				0.173	
Nutrient	K_a	$r(n=6)$	\bar{N}	3 [#] (10%)			K'_a
				$\frac{1}{K_N + \bar{N}}$	P	$\frac{1}{K_P + \bar{P}}$	
DIN × DRP	0.0028	0.997					3.10
DRP	0.21	0.996	66.7				3.48
DIN	0.004	0.966		6.46×10^{-3}			2.66
NO_3^-	0.008	0.940			1.66		
NH_4^+	0.010	0.988				0.140	
Nutrient	K_a	$r(n=6)$	\bar{N}	4 [#] (50%)			K'_a
				$\frac{1}{K_N + \bar{N}}$	P	$\frac{1}{K_P + \bar{P}}$	
DIN × DRP	0.0002	0.986					0.86
DRP	0.036	0.980	186.5				0.83
DIN	0.002	0.974		3.64×10^{-3}			0.85
NO_3^-	0.013	0.974			10.19		
NH_4^+	0.002	0.974				0.0637	

Table 5 Regression for biomass of natural phytoplankton v. nutrient concentration

Nutrient	3 [#] (10%)						
	K_a	$r(n=5)$	\bar{N}	$\frac{1}{K_N + \bar{N}}$	\bar{P}	$\frac{1}{K_P + \bar{P}}$	K'_a
DIN × DRP	0.265	0.981					37.3
DRP	10.90	0.988	30.6				50.1
DIN	0.0085	0.987		0.0165			2.8
NO ₃ ⁻	0.011	0.994			0.42		
NH ₄ ⁺	0.038	0.912				0.431	
Nutrient	4 [#] (50%)						
	K_a	$r(n=5)$	\bar{N}	$\frac{1}{K_N + \bar{N}}$	\bar{P}	$\frac{1}{K_P + \bar{P}}$	K'_a
DIN × DRP	0.0004	0.966					0.51
DRP	0.026	0.883	65.9				0.51
DIN	0.0039	0.980		0.0104			0.44
NO ₃ ⁻	0.012	0.977			11.49		
NH ₄ ⁺	0.0059	0.976				0.0747	

No increase in phytoplankton biomass in 1[#] and 2[#] cultures.

(3) As shown in Table 5, K'_a for 3[#] calculated from DIN was obviously lower than those from DRP and DIN×DRP. This was chiefly because the natural phytoplankton previously lived in P-limited environment had a relative high initial rate of uptake on DRP, thus DRP in 3[#] medium was exhausted rapidly and the algae used P stored in cell for growth, which would cause a relatively great error in calculation with substitution of P for P_i and the K'_a value calculated therefore had an obvious difference.

Effect of domestic sewage on assemblages of phytoplankton population

Ph. and Ch. species were merged into "diatom" for counting, Pl. and Pr. were mixed for inoculation in the way of semi-continuous culture ($D = 1/8 \text{ d}^{-1}$) in four vessels (5L) with media of different nutrient contents. The replenishing rates of nutrients (KH₂PO₄ and NH₄Cl or domestic sewage to seawater) for the four media were as follows respectively: 1[#] 0.47 μmol/L DRP d⁻¹ and 9.7 μmol/L DIN d⁻¹ (N:P=21); 2[#] 1.69 μmol/L DRP d⁻¹ and 9.7 μmol/L DIN d⁻¹ (N:P=5.7); 3[#] 0.47 μmol/L DRP d⁻¹ and 23.3 μmol/L DIN d⁻¹ (N:P=50); 4[#] 1.9% of domestic sewage d⁻¹, i.e., 0.93 μmol/L DRP d⁻¹ and 19.1 μmol/L DIN d⁻¹ or 1.02 μmol/L TDP d⁻¹ and 22.2 μmol/L TDN d⁻¹ (N:P=21). Daily observation of algal growth was carried out for 15 day. Regression analysis was performed for biomass of each species during the period of growth vs. inorganic nutrient concentration and the results were shown in Table 6 and 7, from which it could be seen that:

(1) Generally, there were significant correlations between inorganic N or P and algal growth for diatom and Pr., but a poorer one for Pl.. However, in the culture of 2[#], diatom was not so with nutrients but for NO₃⁻. This result demonstrated that the increase of DRP content had

greater impact on the growth as well as nutrient uptake of diatom and Pl.

Table 6 Regression for growth of phytoplankton population vs. nutrient concentration

Species	Nutrients	1# (N:P=21)		2# (N:P=5.7)	
		K_a	r	K_a	r
Dia. $n=5$	DIN×DRP	0.018	0.975	0.0066	0.886
	DRP	0.238	0.942	0.075	0.768
	DIN	0.0099	0.975	0.012	0.895
	NO_3^-	0.019	0.941	0.029	0.957
	NH_4^+	0.020	0.955	0.021	0.788
Platy. $n=7$	DIN×DRP	0.028	0.823	0.014	0.928
	DRP	0.367	0.887	0.164	0.939
	DIN	0.015	0.814	0.026	0.925
	NO_3^-	0.029	0.690	0.061	0.873
	NH_4^+	0.029	0.885	0.045	0.938
Pro. $n=10$	DIN×DRP	0.043	0.997	0.013	0.912
	DRP	0.473	0.983	0.129	0.992
	DIN	0.022	0.996	0.022	0.986
	NO_3^-	0.058	0.979	0.059	0.952
	NH_4^+	0.036	0.979	0.035	0.994
Species	Nutrients	3# (N:P=50)		4# (N:P=21)	
		K_a	r	K_a	r
Dia. $n=5$	DIN×DRP	0.0070	0.974	0.0066	0.969
	DRP	0.270	0.941	0.193	0.937
	DIN	0.0046	0.972	0.0069	0.974
	NO_3^-	0.011	0.990	0.017	0.978
	NH_4^+	0.0081	0.928	0.012	0.942
Platy. $n=7$	DIN×DRP	0.0094	0.790	0.011	0.848
	DRP	0.359	0.878	0.294	0.894
	DIN	0.0061	0.830	0.011	0.852
	NO_3^-	0.013	0.644	0.027	0.731
	NH_4^+	0.010	0.905	0.018	0.892
Pro. $n=10$	DIN×DRP	0.0125	0.984	0.015	0.910
	DRP	0.380	0.992	0.341	0.983
	DIN	0.0072	0.990	0.015	0.975
	NO_3^-	0.024	0.909	0.049	0.876
	NH_4^+	0.0099	0.985	0.021	0.985

Table 7 Correction for nutrient concentration on Table 6

No.	Species	N	$\frac{1}{K_N+N}$	P	$\frac{1}{K_P+P}$	K'_a	DIN×DRP	DRP	DIN
1#	Dia.	20.2	0.0199	0.56	0.407	2.22	1.45	2.18	
	Platy.	17.4	0.0211	0.54	0.410	3.24	2.44	3.12	
	Pro.	15.3	0.0430	0.53	0.971	1.03	0.74	0.99	
2#	Dia.	19.8	0.0201	1.84	0.267	1.23	0.71	1.22	
	Platy.	17.0	0.0213	1.8	0.267	2.46	1.70	2.48	
	Pro.	15.0	0.0435	1.87	0.422	0.71	0.47	0.64	
3#	Dia.	41.3	0.0140	0.60	0.400	1.25	1.17	1.37	
	Platy.	36.3	0.0151	0.57	0.404	1.54	1.62	1.75	
	Pro.	32.5	0.0247	0.54	0.957	0.53	0.49	0.56	
4#	Dia.	33.5	0.0157	1.00	0.345	1.22	1.06	1.27	
	Platy.	29.4	0.0168	0.99	0.346	1.89	1.72	1.91	
	Pro.	26.5	0.0290	1.01	0.663	0.78	0.67	0.77	

(2) It was known from the comparison of K'_a values that replenishing NH_4^+ was favorable to diatom growth and DRP to Pl., and replenishing domestic sewage mainly stimulated Pr. growth.

CONCLUSION

The mathematical model in this paper was the integrated form of the Monod Equation presupposing that algal growth was approximately at steady state within unit time, and it was applicable to analysis of the semiclosed harbor, the relatively steady water body. The half-saturation constants for growth K_P ($\mu\text{mol/L}$) calculated on the basis of Equation (1) were 5.52 Sk., 1.90 Ch. and 0.46 Pr., respectively. The order of the K_P values explained reasonably the phenomenon in the sea that dinoflagellate red tide developed succeeding to nutrient depletion resulted from diatom bloom. The relative characteristics of algal growth could be understood by comparing the values of the "apparent conditional growth constant" K_a and K'_a . Analysis of the experimental data showed that domestic sewage had both stimulation and inhibition effect on algal growth and the former played a leading role. Domestic sewage was more stimulative on dinoflagellate than on diatom and Pl. when inoculating the mixture of these algae. Because eutrophication in the sea is a phenomenon of short period, it is possible to predict it by evaluation of the parameters based on our mathematical model from continuous observation in situ in the frequent period. The model could be used for explaining the simulation experimental results, showing its reasonableness. We had only made a primary attempt and the model should be further improved.

REFERENCES

Kato, S., Hirobe, H. and Takuaki, M., Bull. Jap. Soc. Sci. Fish., 1985, 51(1):7

Ouchi, A., Bull. Jap. Soc. Sci. Fish., 1982, 48(9): 1245

Ouchi, A., Kusu, Y. and Takayama, H., Bull. Jap. Soc. Sci. Fish., 1983, 49(4): 867

Wu Yudian *et al.*, Fertility of domestic sewage and eutrophication in estuarine harbor, POLMET 88' (Hongkong), 1988, 2:646